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Molecular Dynamics Computer Simulations of Multidrug RND Efflux Pumps

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Abstract: Over-expression of multidrug efflux pumps of the Resistance Nodulation Division (RND) protein super family counts among the main causes for microbial resistance against pharmaceuticals. Understanding the molecular basis of this process is one of the major challenges of modern biomedical research, involving a broad range of experimental and computational techniques. Here we review the current state of RND transporter investigation employing molecular dynamics simulations providing conformational samples of transporter components to obtain insights into the functional mechanism underlying efflux pump-mediated antibiotics resistance in *Escherichia coli* and *Pseudomonas aeruginosa*.

Mini Review Article

I. INTRODUCTION

1.1. Molecular Dynamics Simulations

While the determination of the three-dimensional structure of a protein is a landmark on the way to understand its function, one key element is still missing, and that is the element of motion. Proteins are in an ongoing state of motion easily exceeding mere thermal fluctuation and in most cases this conformational dynamics is the foundation enabling a protein to carry out its physiological function in the first place [1,2]. Part of the molecular mechanical branch of modelling techniques [2], molecular dynamics (MD) simulations numerically investigate the motion of a system of particles under the influence of internal (interactions between atoms) and external forces such as temperature or pressure [3] as well as optional additional forces in steered or targeted MD [4]). A key ingredient of MD simulations is the potential energy function that relates energy to structure using harmonic, periodic, Coulomb and Lennard Jones-like potentials to calculate the forces acting on each particle in the system. Employing Newton's second law of motion MD simulation uses this information to predict each particle's motion during the next few femtoseconds. Repeating this step millions of times, a trajectory of all atoms in the system over time is generated [1-3,5]. Complementing and extending the nearly static experimental 3D data MD simulations bring back for a limited time the element of motion, permitting to cast a glimpse on the dynamics of a (e.g. membrane) protein and its immediate microenvironment at a level of detail not accessible by any experiments today. Moreover, by bringing together a system's components to study their interplay, MD simulations offer a literally synthetic approach of investigation instead of dissecting the system to deduce its functional mechanism.

Since the first MD studies published by Alder and Wainwright more than 50 years ago [6,7], the first MD simulation of a protein carried out by McCammon and co-workers 20 years later [8], the first simulation of a lipid bilayer by Van der Ploeg and Berendsen in 1983 [9], and the first simulation study of a bilayer-embedded membrane protein by Edholm et al. 17 years ago [10], MD simulations have benefited enormously from the impressive advances made in computer and software development, now permitting the investigation of simulation systems of the size of $10^5 - 10^6$ atoms on a nanosecond to millisecond time scale [11-13]. Beyond providing high resolution conformational samples of proteins and other biomolecules, MD simulations have also recently been employed as a tool to compare and categorize proteins, adding internal conformational dynamics as a third level of protein classification next to amino acid sequence and protein structure [14].

A key question of any MD simulation is whether the amount of conformational sampling achieved is adequate for the problem under investigation. Whereas for small individual molecules appropriately long simulations can be performed permitting a sufficient sampling of the available degrees of freedom, for large molecules like proteins only a partial sampling of conformational space is possible today [15]. However, partial sampling can already yield valuable insights into protein function providing e.g. a set of configurations near the X-ray structure, based on which conformational sub-populations comprising the entire reaction cycle can be determined [11,16-19]. Moreover, transportation pathways and interaction sites can be elucidated by analyzing e.g. the dynamics of solvent molecules [20-23]. New mutagenesis candidates can be identified as they undergo for example specific distance changes throughout the reaction cycle [16-19,24] or impacting protein activity [1,11,16,20-22]. To enhance conformational sampling additional forces can be used biasing the simulation in a steered manner [24-34] or the simulation can be performed running several independent copies of the same system differing only in the random seed numbers used in generating the starting velocities. While stating the respective simulation approaches

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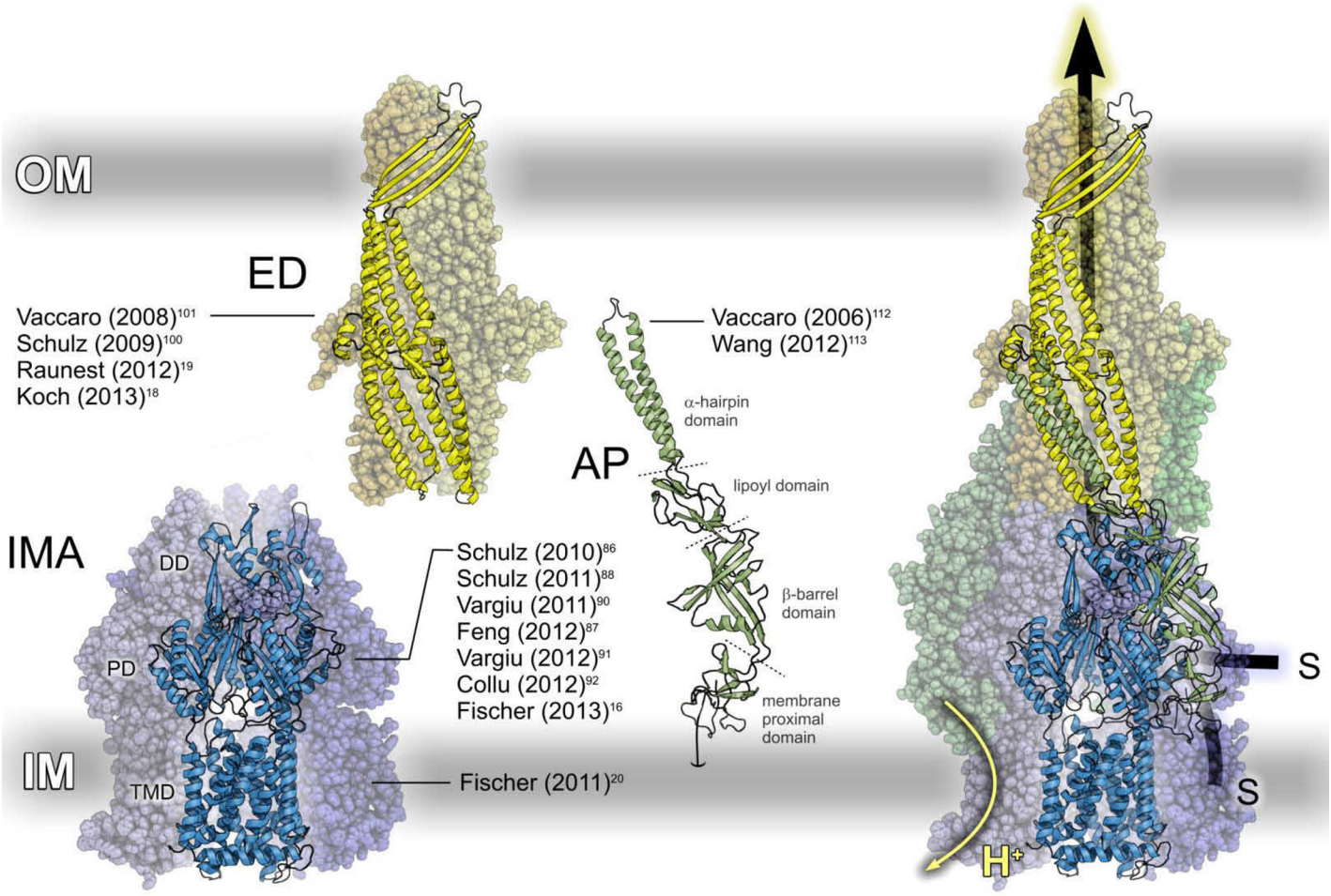

Figure 1. RND efflux pumps comprise three different components (left) assembling into a functional complex (right). Using the proton concentration gradient over the inner membrane (IM) the inner membrane proton / substrate antiporter (IMA) acts as engine and active transporter of the efflux pump, expelling substrates (S) out of the cell via the access-regulated efflux duct (ED) in the outer cell membrane (OM). In the assembled pump IMA and ED are coupled by an inner membrane-anchored adaptor protein (AP), whose actual stochiometry and location in the assembled pump is not known for all RND efflux transporters. To visualize the structure of the assembled IMA-ED-AP complex we used a docking model based on biochemical cross-linking data [41]. The references in the figure represent simulation studies of the respective efflux pump component discussed in this review.

employed in the studies presented in this review, we refer the reader to the original publications for further-going in-depth information and discussion of the individual methodologies, approximations made and their adequateness for the questions investigated.

1.2. RND Efflux Pump-mediated Antibiotics Resistance

The discovery, development and clinical exploitation of antibiotics count among the most significant medical advances in history. However, antibiotics lose their efficiency after a period of months to years [35-37], eventually producing new strains of resistant bacteria, as the continuous application of antibiotics wipes out the cells in a bacteria population sensitive to the drug given. At the same time this effect creates perfect survival conditions for the fraction of bacteria immune to the pharmaceuticals applied. With old antibiotics losing their efficiency faster than new ones can be developed [38], a detailed understanding of the molecular basis of microbial multi-drug resistance is paramount for modern biomedical research. The main mechanisms of action underlying antibiotics resistance include the alteration of the drug, the alteration of the drug target as well the reduction of antibiotics concentration inside the bacterium by lowering influx into and/or enhancing the extrusion out of the organism [39,40].

A major way by which Gram-negative bacteria achieve an increased extrusion is through an over-expression of multi-drug efflux pumps of the resistance nodulation division (RND) protein super family [42], preventing drug access to the target molecule [43,44]. RND transporters function as transiently assembled protein complexes constituting (a) an inner membrane proton / substrate antiporter, functioning as engine and active transporter of the assembled pump (figure 1, IMA); (b) an access-regulated outer membrane channel acting as efflux duct for substrate trafficking (figure 1, ED) and (c) an inner membrane-anchored adaptor protein (figure 1, AP) coupling IMA and ED, enhancing pump activity [45]. Whereas crystal structures have recently become available for all components of three different but structurally homologue RND efflux pumps in *Escherichia* coli (AcrAB-TolC and CusBA-C) [41,46-57] and *Pseudomonas aeruginosa* (MexAB-OprM) [41,58-60], the structure of the assembled pump is unknown. The visualization of the assembled IMA-AP-ED complex in figure 1 shows a docking model based on biochemical cross-linking data [41]. Whereas this model comprises three APs interacting with IMA and ED, recent studies suggest that MexA and AcrA form a funnel-like hexamer when binding to their respective EDs [61-63] similar to the IMA-AP crystal structure of the heavy metal efflux transporter CusBA [57].